DESIGN AND EVALUATION OF ITRACONAZOLE LOADED SOLID LIPID NANOPARTICULATE SYSTEM FOR IMPROVING THE ANTIFUNGAL THERAPY

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ABSTRACT
The objective of the study was to design and evaluate Itraconazole loaded solid lipid nanoparticles (SLNs) drug delivery system, where Itraconazole nanoparticles with suitable size ranges are expected to improve the therapeutic efficacy and reduction of toxicity of this broad spectrum antifungal agent. Components of the SLNs were lipid (palmitic acid) and surfactants (Pluronic F127 and Tween 40). The Itraconazole loaded nanoparticles were prepared by microemulsion dispersion method. Experiments were carried out with optimized ratio of excipients, where drug-lipid ratio and surfactant-cosurfactant ratio (Km) were varied to optimize the formulation characteristics. The effects of dispersion media, its pH, ionic content, etc. were investigated to optimize the SLNs production. Particles size analysis and zeta potential measurements were done using Malvern Mastersizer Hydro 2000G. The particles were also subjected to DSC, IR and XRD analyses. The *in vitro* drug release profile from nanoparticles was found to prolong up to 12h. Kinetic analysis of release indicated that nanoparticles formed were matrix in nature, in which Itraconazole dispersed uniformly. Optimized formulations were found to have a lipid-drug ratio of 1.5:1 and prepared at a Km ratio of 1:2 to maximize drug loading, modulate release and minimized particle size. The microemulsion mediated nanoparticle preparation methodology ensured high drug loading (ca. 80%), low and narrow size distribution and provided a reproducible and fast production method. The study elaborates on the feasibility and suitability of lipid based colloidal drug delivery system, employing optimize design to develop a clinically useful nanoparticle system with targeting potential.

Keywords: SLN, Itraconazole, Pluronic, micrormulsion, optimization, XRD, DSC.

INTRODUCTION

Solid lipid nanoparticles (SLN) are colloidal carriers, which were developed at the beginning of the 1990s as an alternative system to the existing traditional carriers (emulsions, liposomes and polymeric nanoparticles), especially for the delivery of lipophilic compounds (Hu *et al*., 2004; Lim *et al*., 2004; Tabbat *et al*., 2004). These types of colloidal drug delivery systems are useful for different routes of administration (Muller and Keek, 2004; Venkateswarlu and Manjunath, 2004; Wissing *et al*., 2004; Kim *et al*., 2005). They show some potential advantages like drug leakage during storage and insufficient drug load. To overcome the limitation of SLN, nanostructured lipid carriers (NLC) have been developed (Muller *et al*., 2002a). Both carrier types are submicron size particles (50-1000 nm) and are based on solid lipids but they can be distinguishing by their inner structure. SLNs consist of solid lipids while NLC are made of solid matrix entrapping variable liquid lipid noncompartments (Muller *et al*., 2002b). NLC is also called as an upgrade of the solid lipid nanoparticles even though SLNs is still intended to indicate the nanostructered lipid carriers, creating no clear differentiation. The NLCs have mainly been investigated in the topical and dermatological preparations (Muller *et al*., 2002) in the delivery of clotrimazole (Souto *et al*., 2004; Souto and Muller, 2006), ketoconazole (Souto *et al*., 2005), other antifungal imidazoles (Souto and Muller, 2006) and ascorbyl palmitate (Uner, 2006). The successful implementation of lipid nanoparticles for drug delivery depends on their ability to penetrate through several anatomical barriers, sustained release of their contents and their stability in the nanometer size. SLN offer unique properties like small size, large surface area, interaction of phases at the interfaces, and are attractive for their potential to improve performance of pharmaceuticals, neutraceuticals and other materials (Goodman and Gilman's, 2002). SLNs are drawing major attention as novel colloidal drug carrier for intravenous applications (Vyas and Khar, 2002).

The SLNs are submicron colloidal carrier, which is composed of physiological lipid, dispersed in water or in an aqueous surfactant solution. It has advantages of good tolerability, scalability to large-scale preparation, excellent biocompatibility and protection of incorporated drugs against chemical/ enzymatic degradation. If systematically investigated, SLNs may open new avenues in research and therapy (Ghorab Mamdouh *et al*., 2005).

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Selection of suitable lipids and surfactants for formulating Solid Lipid Nanoparticles (SLNs), have made it possible to cross lipoidal Blood Brain Barrier (BBB) and deliver the desired drugs to highly restricted cerebral site. Further, numerous applications have been reported in literature and excellent reviews covered the clinical applications of lipid nanoparticles. As such they hold a great promise in materializing legendary Ehrlich′s dream of a Magic Bullet (Mehnert and Madder, 2001; Sarciqux *et al*., 1995; Yang *et al*., 2003; Chi Sang and Rhee Yun, 2001). In the present investigation, the focus was on the development of suitable colloidal dispersed system (solid lipid nanoparticles) of itraconazole, along with physiologically compatible lipid, palmitic acid and FDA approved surfactant, Pluronic F-127 (Prakobvaitayakit and Nimmannit, 2003). Itraconazole is a weakly basic (pKa = 3.7) and highly hydrophobic (octanol/water partition coefficient at pH = 8.1, logP = 5.66) triazole derivative (Chasteigner *et al*., 1996). Its aqueous insolubility resulted large interindividual and intraindividual variation of its oral bioavailability (Heykants *et al*., 1989).

Therefore, in this investigation, an attempt has been made to prepare homophasic or heterophasic colloidal dispersed system from suitable rate modulating macromolecules and surfactants, to entrap itraconazole, and to study different formulations and processing characteristics like particle size determination, measurement of zeta potential, x-ray diffractometry (XRD) (Laggner, 1999), differential scanning colorimetry (DSC) (Meyer *et al*., 1992), atomic force microscopy (AFM) (Drake *et al*., 1989) etc of the system. The solid lipid matrix based systems such as nanoparticles/lipospheres or their suspension form is envisaged as the target formulation. Such a dispersed system would be expected to improve the bioavailability, stability and make it more useful against broad spectrum mycoses. The toxicity profile of the potent drug is also expected to be reduced significantly when delivered in colloidal system, presumably due to reduction in effective dose and better distribution profile.

Table 1. Master Batch formulae

Code	Composition (%)					Dispersion Medium	Vol. of Dispersion medium (ml)
	Palmitic acid (Lipid)	Itr (Drug)	Pluronic F127 + Tween 40 (Surfactant)	Butanol+ Ethanol (co-surfactant)	Water		
F1	18.27	11.67	16.50	34.55	18.98	d. Water	50
F2	18.05	11.07	16.96	34.41	19.49	d.water +0.1N NaOH	50
F3	18.10	11.03	16.90	34.53	19.42	d.water +0.1N Kcl	50
F4	17.70	10.62	17.59	34.59	19.48	d.water +0.1N CaCI2	50
F5	18.32	10.92	16.99	34.22	19.53	d.water+0.1NNaOH+0.1NCaCl2	50
F6	18.07	10.72	17.10	34.57	19.51	0.1M CaCI2 + PBS pH 5.4	50
F7	18.09	10.85	17.61	33.89	19.54	0.1 N HCI	50
F8	17.27	11.24	18.22	33.37	19.88	0.1 N NaOH	50
F9	17.53	10.78	18.48	34.24	19.95	0.2M CaCI2+0.1N NaOH	50
F10	17.48	11.53	18.06	33.56	19.34	0.05M CaCI2+0.1N NaOH	50

d.water – double distilled water, PBS – Phosphate buffer soln.

Table 2. Particle size, Zeta potential, drug content (%) and drug entrapment (%) of different formulation

Formulation Code	Particle Size d.nm	PdI	Zeta Potential mV	Drug content (%)	DEE (%)
F1	380	0.715	-28.5	9.97	24.92
F2	521	0.615	-24.1	8.79	21.97
F3	618	0.863	-23.7	8	20
F4	756	0.878	-19.3	6.13	15.32
F5	515	0.967	-21.4	7.58	18.95
F6	426	0.993	-26.5	7.27	18.17
F7	897	0.615	-16.8	4.58	11.45
F8	442	0.856	-27.4	7.13	17.82
F9	537	0.673	-24.9	7.42	18.55
F10	527	0.941	-19.8	7.15	17.87

Table 3. *In vitro* cumulative drug release (%) of different formulation from rat ileum

Time (Min)	% CDR									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
15	24.96	17.73	17.75	8.96	7.58	10.62	3.65	5.793	2.41	11.70
30	27.77	21.31	21.11	11.77	10.01	13.43	5.46	7.75	4.39	13.14
60	31.18	24.51	24.57	14.54	12.78	16.35	7.89	10.52	5.54	15.98
120	36.17	27.35	28.90	18.97	17.26	20.36	9.97	15.64	9.69	19.18
180	41.16	34.45	34.26	23.69	20.03	24.80	13.36	20.50	15.00	22.73
240	46.57	40.14	39.46	27.99	22.80	29.37	16.21	25.35	21.47	25.93
300	51.56	46.18	43.96	32.84	25.22	34.09	20.29	30.34	26.57	28.78
360	56.56	52.93	48.29	37.83	28.33	39.08	25.62	35.06	30.86	32.33
420	62.24	57.56	52.8	42.27	31.09	43.80	30.69	39.78	35.60	34.82
480	67.24	61.47	56.61	47.13	33.51	48.79	34.58	44.35	40.12	38.37
540	72.09	64.67	60.59	51.43	36.28	53.37	38.81	49.62	45.09	42.99
600	76.26	67.51	64.06	55.04	38.70	57.53	42.28	52.55	49.60	45.13
660	79.59	69.65	66.83	57.95	40.43	61.42	45.13	56.29	53.43	47.26
720	82.92	71.78	69.77	60.32	41.81	63.78	46.87	60.31	56.44	48.33

Although various methods have been reported in literature, the followed method of solid lipid nanoparticle (SLN) preparation is a thermodynamically spontaneous technique of SLNs preparations. It is also less equipment intensive and has good commercial scale-up feasibility of the master formula. The formulations have been developed keeping in view their reproducibility to characterize the effect of various formulations and processing parameters on the quality and performance of lipid nanoparticles (Alonso and Torres, 2002). It is expected that this type of itraconazole loaded lipid nanoparticulate system could be clinically effective in better management of systemic and ocular inflammation with greater degree of safety and efficacy.

MATERIALS AND METHODS

Materials

Itraconazole (Itr) was obtained as gift sample from Hetero Drugs Pvt .Ltd, Hyderabad and surfactant (Pluronic-F 127) and Lipid (Palmitic acid) were received as gift samples from Sun Pharmaceuticals Ltd., Baroda. Acetonitrile, dichloromethane and all other reagents were procured commercially and used as obtained. Double distilled water was used in the experiment.

Method

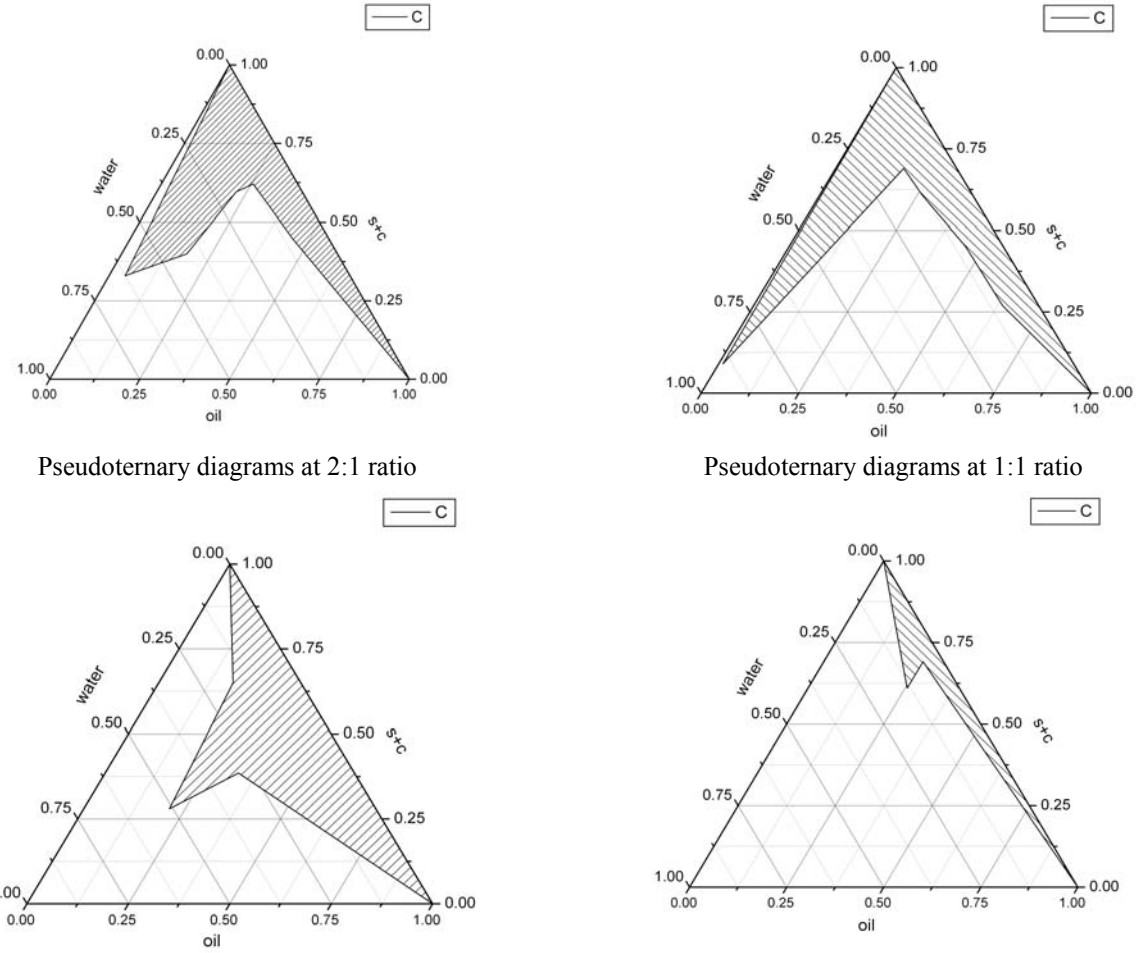
The maximum amount of drug loaded in the melted lipid were 1:1.5. Then the molten lipid mixture was made a clear microemulsion above the melting temperature of the lipid by the water titration method. Briefly, molten lipid and surfactants and/or cosurfactants were mixed in different proportions ranging from 0% to 100% lipid and each mixture was titrated with water till the clear mixture turned turbid. The appearance of the turbidity was taken as the limit of the microemulsion zone. The surfactant to cosurfactant ratio (Km) was optimized to obtain the microemulsion with the broadest clear zone in the pseudo-ternary phase diagram (fig. 1). The optimized Km ratio was found to be 1:2.

In the method of preparation, weighed accurately palmitic acid and itraconazole (1.5:1) were taken in Eppendorf tube. Then weigh accurately the pluronic F-127 and Tween 40 (w/w) in the ratio of 1:1 and poured Eppendorf tube. Then added cosurfactant (butanol+ethanol = 1:1 w/w) and water (w/w) in eppendorf tube. The tube was placed in a water bath, which was heated at 80^{o}C for 5 minutes for melting of solid substance. The clear warm microemulsion was then developed.

Solid Lipid Nanoparticles (SLNs) preparation via microemulsion method (Capek, 2004)

The warm microemulsion was dispersed in the different types of aqueous media in different ratio of microemulsion to aqueous medium ranging from 1:25, 1:50, 1:75 and 1:100. The 1:50 ratio was found to be optimum based on the visual appearance and particle size distribution study.

The dispersion medium exposed both chilled and hot states to ascertain the effect of variation in the temperature on dispersion medium and to determine process variables. It was found that the chilled condition resulted lipid aggregates. On the other hand, the dispersion medium was kept hot during dispersion and then slowly cooled to room temperature with stirring at 2500rpm for 30 minutes yielded a nanosuspension. This nanosuspension was homogenized using high-speed homogenizer (Polytron 1200E) at 30000 rpm for 5 minutes. The various formulations prepared by varying drug, lipid, surfactants, cosurfactants and dispersion medium are given in table 1.



Pseudoternary diagrams at 2:1 ratio

Pseudoternary diagrams at 1:1 ratio

Pseudoternary diagrams at 1:2 ratio

Pseudoternary diagrams at 3:2 ratio

Fig. 1: Pseudoternary diagram at different ratios.

Particle size analysis

Particle size was determined by using Malvern Mastersizer Hydro 2000G at 25°C using disposable sizing cuvette. The count rate was kept at around 200 kcps with varying duration greater than 50s. The dispersant used was water and its RI (1.33), viscosity (0.8872 cP) and Dielctric constant (78.5) were kept constant for all determinations. 1 ml sample was taken from each formulated nanosuspension and dispersed with 10ml of double distilled water. The samples were ultrasonicated for 5 min prior to size determination to measure the primary particle size. Then the sample was taken in disposable sizing cuvette and placed in the instrument for size and zeta potential measurements.

Drug content of the dispersed formulation

The loading efficiency of itraconazole in the nanoparticles was determined specrophotometrically at 263nm. 5ml nanosuspension was taken and diluted 25ml with acetonitrile. 1ml was taken from that solution and diluted to 25ml. The concentration was measured by UV spectrophotometer at 263nm. The amount of drug entrapped in the nanospheres was then calculated.

DEE (Drug Entrapment Efficiency)

$$\text{DEE} = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \text{X}\,100$$

Drug Release Study

Release studies were carried out for all the formulations by using the HIMEDIA DM70 dialysis membrane (pore diameter of 2.4 nm and cut off of 12-14 kD) in phosphate buffer (200ml, pH 7.4) containing 2% SLS. The membrane was activated by incubating in 5% EDTA solution for 0.5h and then in boiling water for 1h prior to use. Earlier, one side of the membrane was tied with a thread and nanosuspension (10ml) was then placed inside the membrane, the other side was tied properly, and placed in 200ml release medium in a beaker with stirring

for 24h. 2ml of the aliquot was withdrawn at different predetermined intervals. The required dilutions were made with dissolution medium and the solution was analyzed for the drug content spectrophotometrically at 263nm. Equal volume of the dilution medium was replaced after each withdrawal to maintain sink condition. The release studies were carried out in triplicate for all the formulations and the average, SD and SEM values were calculated. From this, percentage drug release was calculated and this was plotted against function of time to study the pattern of drug release.

Measurement of Zeta Potential

Zeta Potential was determined by using Malvern Mastersizer Hydro 2000 G. Nanosuspension sample (1ml) was taken and dispersed in double distilled water. To prevent the agglomeration, the dispersed solution was placed for 5 minutes in ultra sonicator bath. Then the sample was taken in the glass cuvette and zeta potential was measured by using Malvern Mastersizer Hydro 2000G.

Permeation Study

Rat intestinal tissue was used to determine the drug permeation profile. The animals were faster overnight before the study and given water *ad libitum*. In this method, 5cm of intestinal tissue (ileum) was cut off from the anaesthesized rats and washed with Tyrode's solution. Then, one side of the tissue was tied with surgical suture and introduced 0.5ml of the nanosuspension inside the tissue. Another side was then tied carefully and placed in a beaker contain 40 ml of phosphate buffer pH 7.4 containing 2% SLS. The tests were carried out for 1h with constant magnetic stirring while maintaining aeration throughout the study. 5ml of aliquot was withdrawn at different predetermined intervals from the release medium. The required dilutions were made with phosphate buffer and the solution was assayed for the drug content spectrophotometrically at 263nm. 5ml of the dilution medium was replaced in the vessel after each withdrawal to maintain sink condition. Three studies were carried out in triplicate for all the formulations and the average, SD and SEM values were calculated. From this percentage drug permeation was calculated and was plotted against function of time to determine the pattern of drug permeation.

Crystal properties

Polymorphic composition and degree of crystallinity were evaluated by X-ray powder diffraction (XRPD). X-ray powder diffraction experiments were carried out using a simens D-5000 X-ray diffractometer with Co Kα radiation (λ = 1.7890 Å) at scanning speed of S^{-1} 2θ over a range of 3-50^{o}. Silicon was served as the internal standard. Diffrac AT software was used to display the X-ray diffraction patterns.

Differential scanning calorimetry

Samples were analysed for crystalinity of lipid by differential scanning calorimetry (DSC), with a Mettler-Toledo DSC 822^{o} instrument (Mettler-Toledo GmbH Analytical, Schwerzenbach, Switzerland) using nitrogen gas. The sample (4 mg) was poured in a 40 µl Al crucible, which was then sealed. The sample was kept at 25^{o}C for 10 min, and heated from 25 to 250^{o}C at a scan rate of 5^{o}C/min.

RESULTS AND DISCUSSION

Particle size determination

Particle size measurement with Malvern mastersizer was performed after dilution of the samples, surprisingly it was found that the systems preserved their colloidal particle size between 380-897nm. It was found that the particle size was less (380nm) when the distilled water used as a dispersion medium. The particle size was bigger in case of the other electrolyte medium. But the observed particle size of all of the investigated system was in the colloidal ranges (table 2).

Drug content of the dispersed formulation

The drug content (%) of the all dispersed systems were variable. The drug content was found more in case of distilled water than the other electrolyte solution (table 2).

DEE (Drug Entrapment Efficiency)

It was found that the drug entrapment efficiency was also varied as per the dispersion medium. The entrapment efficiency (%) was high in case of distilled water (table 2).

Drug Release Study

It was found that the optimum drug release was observed from the formulation (F1) was up to 12h. About 80% drug released from nanoparticles while dispersed in distilled water (table 3).

Measurement of Zeta Potential

The surface charge of the particle was changed by altering the dispersion medium (table 2). In case of the distilled water the zeta potential was found to -28.5 where the zeta deviation was 7.5. As we know the standard zeta potential was +40 to -40. So the zeta potential -28.5 which was near the range contains the very small particle size in the formulation (F1). The variation of zeta potential was related with nature of dispersion medium.

Permeation Study

The of cumulative drug (%) permeates after 1h was found higher in F1, where distilled water was used as the dispersion medium. The percentage drug permeation through rat intestinal tissues of different formulations (F1–F10) were variable (fig. 2).

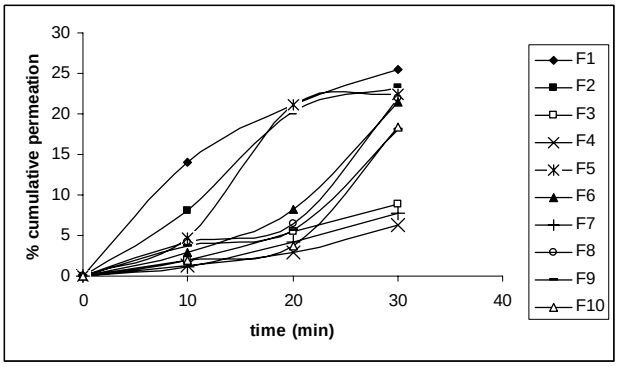


Fig. 2: Permeation profile of the formulations

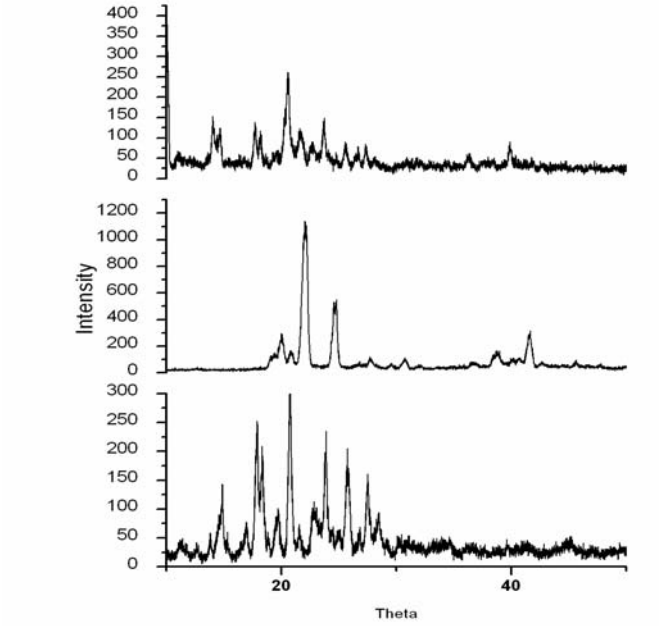


Fig. 3: X-ray diffractometry of the drug (below), lipid (middle) and F1 (top).

Crystal properties

The crystalline properties of drug was reduced by forming in the SLNs, however the drug retaines its crystallinity and has not completely changed into amorphous form (fig. 3). The decreasing intensity of the XRD peaks may be attributed to lower drug content (around 40%) in the nanoparticles. There is no appreciable change in the position of the peak with respect to θ values. Due to comparatively high drug content (approx. 40%), it is expected that the drug did not get anough space for dilution amorphization within the nanomatrix of SLNs. Coupled with this relatively fast cooling rate (from 85°C TO RT) might not allow conversion into amorphous form.

As regards the lipids, there has been shift/disappearance of few of the peaks in the X-ray diffractogram which might be an indication of formation of different polymorph of the lipid, presumably due to the heating and cooling process.

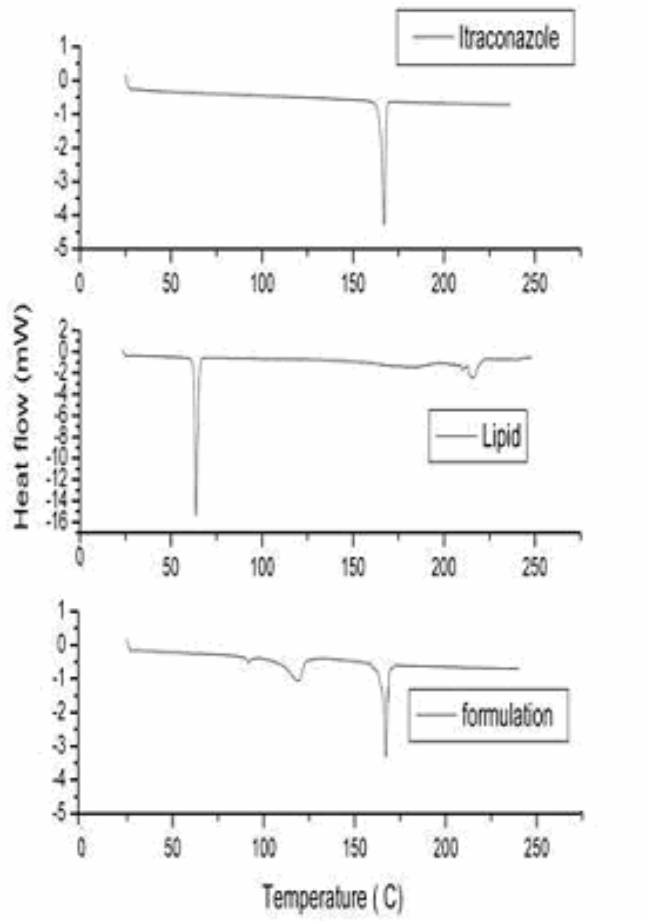


Fig. 4: Differential scanning calorimetry of the drug, lipid and formulation (F1)

Overall, the drug loaded SLNs retain the crystallinity of drug, which is indicative of a heterogenous drug distribution in the lipid matrix. This might be the cause for a steady release since as per classical matrix dissolution theory, the drug must dissociate from their crystal structure prior to diffusion through matrix. The initial brust release (upto 31%' F1) at 1st h may be attributed to the drug crystal embedded to SLN surface and their dissolution enhancement due to co-existence of hydrophilic surfactant pluronic F-127.

Differential scanning calorimetry

The DSC thermogram of pure drug, lipid and drug loaded SLN showed marked variations in their thermal profiles (fig. 4). The melting endotherm of lipid as well as the degradation shoulders disappeared in the formulation and they are appearing to a shift of Tg towards lower temperature in the SLNs. This might be due to the presence of drug and Pluronic F127 acting as plastisizer,

also the appearance of different polymorph of lipid, as a consequence of thermal cycling during SLN preparation. However the drug melting peak was retained almost same position like pure drug indicated further crystallinity of itraconazole which was complimentary to the same finding of XRD. The presumed polymorphic modification of lipid might be instrumental to provide a steady but almost complete release upto 12h study.

CONCLUSIONS

The formulations can be economically manufactured from relatively cheap raw material, like palmitic acid, Pluronic F127, Tween 40 etc. Lipid nanoparticles with suitable and desired characteristics may be prepared by microemulsion dispersion technique. The release profiles of Itraconazole from the SLNs are amenable to slow delivery of the drug to afford at least twice daily administration.

The formulation profile of nanoparticles of Itraconazole was mediated through pseudo-ternary phase diagrams for developing the microemulsion concentrate before dispersing it in suitable quantity of the aqueous congealing phase (fig. 1). The optimized Km ratio was 1:2 and the optimized drug-lipid ratio was 1:1.5. The microemulsions have inherent droplet size of the internal phase (molten lipid in this case) in the range < 200nm, which makes the ideal process for preparing nanoparticles of low and narrow size range.

The developed SLNs offers the advantages of high drug-lipid ratio, drug loading, minimal particle size and size-distribution and a moderate zeta potential of the particles.

The nanoparticulate colloidal drug delivery system of Itraconazole prepared from palmitic acid and Pluronic (F 127) surfactant was expected to provide the clinician with a new choice of an economical, safe and efficient regimen for the management of systemic fungal infection or against broad spectrum mycoses.

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